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Inhibition of platelet-derived growth factor-induced mesangial cell proliferation by cyclooxygenase-2 overexpression is abolished through reactive oxygen species

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Abstract **Proliferation of mesangial cells (MC) is an early event in many forms of glomerulonephritis. We have previously shown that platelet-derived growth factor (PDGF)-induced proliferation of MC was inhibited by the overexpression of cyclooxygenase-2 (COX-2). Since reactive oxygen species (ROS) are important mediators of mitogenic signaling, we evaluated the role of ROS in the COX-2 induced growth arrest in MC. We demonstrate that ROS are reduced in COX-2 overexpressing MC. Intracellular elevation of ROS restored PDGF-induced proliferation, while the expression of the cyclin-dependent kinase inhibitors $p21^{cip1}$ and $p27^{kip1}$ were decreased in these cells. The data suggest that COX-2 decreases ROS formation which consequently leads to the PDGF-induced inhibition of MC proliferation.**

Keywords: Reactive oxygen species; Cyclooxygenase-2; Mesangial cells; CDK inhibitors; PDGF; Proliferation; Cell cycle

1. Introduction

Proliferation of renal mesangial cells (MC) is an early event in proliferative glomerulonephritis (GN) which may initiate processes eventually leading to glomerulosclerosis [1]. In experimental GN platelet-derived growth factor (PDGF) is an important inducer of MC proliferation. Interfering with PDGF ameliorates GN [2]. Enhanced generation of reactive oxygen species (ROS) and their role as regulators of intracellular signaling in mitogenic pathways was also demonstrated in human and experimental GN [3–6]. Brar et al. demonstrated the requirement for ROS in PDGF-induced growth in airway smooth muscle cells [7]. ROS which are generated directly in response to PDGF seem to be important in the subsequent signaling events leading to proliferation.

The rate-limiting enzymes in the formation of prostaglandins are the cyclooxygenases [8]. Two cyclooxygenase isoforms are currently known. Cyclooxygenase-1 (COX-1) is constitutively expressed in many tissues and is responsible for the physiological functions of prostaglandins. In contrast, cyclooxygenase-2 (COX-2) is an immediate early response gene which is rapidly induced by proinflammatory cytokines and growth factors. COX-2 gene expression is increased in diseases associated with proliferation such as cancer and rheumatoid arthritis [9,10]. We recently demonstrated, however, inhibition of PDGF-induced proliferation of MC by stable overexpression of COX-2 through upregulation of the cyclin-dependent kinase (CDK) inhibitors $p21^{cip1}$ and $p27^{kip1}$ [11].

Since MC directly generate ROS independently of infiltrating cells [12], the present study examines the role of ROS in the COX-2 mediated inhibition of PDGF-induced proliferation of MC. In growth inhibited COX-2 overexpressing MC (COX-2+ MC), ROS concentration is decreased. Intracellular increase of ROS through menadione abolished growth inhibition in COX-2+ MC through downregulation of the CDK inhibitors $p21^{cip1}$ and $p27^{kip1}$. This study suggests that a distinct ROS level in MC is necessary for PDGF-induced proliferation and COX-2 seems to influence MC proliferation by triggering ROS.

2. Materials and methods

2.1. Cell culture

Cell lines used in this study were characterized previously [11]. An *Eco*RI/*Kpn*I full length PCR construct of rat COX-2 was cloned into the mammalian expression vector pcDNA3.1-Zeo (Invitrogen). For transfection, 2×10^5 rat MC between passage 15–18 were seeded in RPMI-1640 containing 10% FCS (Invitrogen). A mixture of 5 μg COX-2 plasmid or empty pcDNA3.1-G418 plasmid for control cells and 20 μg/ml Lipofectine (Life Technologies) in RPMI-1640 were added to 70–80% confluent MCs for 6 h at 37 °C in 5% CO_2. Cells were maintained in RPMI-1640 containing 10% FCS for 48 h before selection was started by adding 200 μg/ml zeocin (Invitrogen) or 400 μg/ml G418 (Invitrogen) for two weeks. Single cell clones of stable transfected MCs were established further through limiting dilution, and were cultured in 10% FCS RPMI-1640 supplemented with 100 μg/ml zeocin (COX-2+ MC) or 200 μg/ml G418 at 37 °C (VC-MC) in 5% CO_2. The two pcDNA3.1 vectors were used to avoid cross contamination of control (VC) and COX-2+ MCs.

2.2. Measurement of reactive O_2

Reactive O_2 measurement was performed through the ROS dependent hydrolysis of CM-H_2DCFDA (Molecular Probes) and subsequent fluorescence detection. Serum-deprived confluent COX-2+ and

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Abbreviations: COX-2, cyclooxygenase-2; ROS, reactive oxygen species; PDGF, platelet-derived growth factor; CDK, cyclin-dependent kinase; COX-2+, cyclooxygenase overexpressing cell line; MC, mesangial cells; GN, glomerulonephritis

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control (VC) MC or appropriate cells which were additionally treated with 5 μM menadione or 50 ng/ml PDGF for 1 h were supplemented for the last 20 min with 2.5 μM H_2DCFDA. Then the cells were trypsinized and washed twice in PBS. H_2DCFDA fluorescence was determined by using 0.5×10^6 cells per ml in PBS with the FL1 channel (excitation 488 nm; emission 515–545 nm) of a fluorescence-activated cell sorter calibur (Becton Dickinson).

2.3. Cell proliferation assay

Cells (5×10^3 cells/well) were plated on 96-well plates (Nunc) and maintained in RPMI-1640 media supplemented with 10% FCS over night. Subsequently, cells were incubated in serum free RPMI-1640 media for 24 h. DNA synthesis was measured by [^{3}H] thymidine incorporation. 2 μCi/ml [^{3}H] thymidine (90 Ci/mmol; Amersham Pharmacia Biotech) was added to the serum free media for further 24 h. [^{3}H] thymidine incorporation of the appropriate control group was compared to cells that were additionally treated with 50 ng/ml rat PDGF-BB (R&D Systems), 5 μM menadione or with the combination of 50 ng/ml rat PDGF-BB and 5 μM menadione for 24 h. At the end of the incubation period, cells were washed twice with 1 × PBS and were then trypsinized. The cell suspensions were subsequently harvested onto a filterpaper (Whatman) using an automated cell harvester (Dynatech) before [^{3}H] thymidine incorporation was measured in a β-scintillation counter (Packard).

2.4. Cell counting

COX-2+ and control (VC) MC were seeded in 24-well plates (3×10^4 per well) and maintained in RPMI-1640 medium supplemented with 10% FCS over night. Subsequently, cells were incubated in serum free RPMI-1640 media for 24 h. Cells in triplicate were treated either with 50 ng/ml PDGF-BB or 5 μM menadione alone or with the combination of 50 ng/ml rat PDGF-BB and 5 μM menadione for 24 h. At the end of the incubation period, cells were washed twice with 1 × PBS and were then trypsinized. Cells were resuspended in 100 μl serum free RPMI-1640 media and subsequently counted in a Neubauer chamber.

2.5. Cell viability assay

Cell viability was measured by the CytoTox® non-radioactive cytotoxicity assay obtained from Promega according to the manufacturer's recommendations.

2.6. Western blot analysis

Cells were washed with 1 × PBS and lysed in 1 × cell-lysis buffer (150 mM Tris–HCl, pH 6.8, 6.6% SDS). Equal amounts of protein were treated with 1/4 vol reducing buffer (50% mercaptoethanol, 50% glycerol) as well as 1/5 vol gel loading buffer (42.5% glycerol, 0.05% bromphenol blue), and samples were boiled for 10 min. The solution was loaded onto a 12% polyacrylamide SDS gel and electrophoresed at a constant current of 20 mA for 4 h. A molecular-weight marker (14.3–220 kD, Amersham Pharmacia Biotech), was run in parallel. After completion of electrophoresis, proteins were electroblotted semi-dry (blotting buffer: 25 mM Tris, 200 mM glycine, 20% methanol) for 1 h at 1 mA/cm^2 onto a PVDF membrane (Hybond ECL, Amersham Pharmacia Biotech). The membrane was blocked in 5% non-fat dried milk in washing buffer (1 × PBS, 0.1% Tween 20) for 1 h at room temperature, then incubated for another hour with the primary antibody in the same buffer. Both primary antibodies (anti human p27^{kip1}, Cell Signaling; anti human p21^{cip1}, BD-Biosciences) were used at a dilution of 1:1000. After rinsing the membrane in washing buffer for 2 × 10 min, the p21^{cip1} blots were treated with an anti-mouse-IgG antibody conjugated to alkaline phosphatase (Southern Biotechnology) at a concentration of 1:3500 for 1 h at room temperature. The p27^{kip1} blots were treated with an anti-rabbit-IgG antibody conjugated to HRP (Southern Biotechnology) under the same conditions. Detection of the alkaline phosphatase activity was performed with CDP-Star (Tropix) in assay buffer (10 mM Tris–HCl, pH 9.6, 150 mM NaCl, 50 mM $MgCl_2$) and detection of the HRP activity was performed with a highly sensitive substrate (Visualizer™, Upstate). Both substrate solutions were used according to the manufacturer's recommendations. Chemoluminescence detection of the blots as well as densitometric evaluation were performed with the FluorS imager system (BioRad). Reincubation of the blots against β-actin (anti mouse β-actin 1:3000; Sigma) were performed to account for small protein loading and transfer variabilities.

2.7. Statistical analysis

The data are presented as the means ± S.D. Statistical significance between different groups was first tested with the non-parametric Kruskal–Wallis test. Individual groups were subsequently tested using the Wilcoxon–Mann–Whitney test. A P value <0.05 was considered significant.

3. Results

As demonstrated in Fig. 1A ROS concentrations in COX-2+ MC are strongly decreased when compared to the mock-transfected control (VC) MC. In Fig. 1B and C, the effect of menadione, a vitamin-K derivate that penetrates the cells and intracellularly generates superoxide, on COX-2+ and control (VC) MC ROS levels were examined. To test, whether menadione could increase cellular ROS levels, quiescent COX-2+ and control (VC) MC were treated with 5 μM menadione for 1 h in serum free RPMI-1640 cell culture media. Menadione strongly increases ROS levels of both cell-lines compared to the appropriate cell-line cultured under serum free conditions. To exclude potential toxic effects of the ROS donor menadione on the cell lines used, cell viability assays were performed. Both cell lines show a viability of 80% when cultivated in serum free RPMI-1640 media only and a viability of 77% in the presence of 5 μM menadione. Therefore, the effects are unlikely due to menadione toxicity. Since PDGF increases ROS levels in other cell systems [7,19] quiescent COX-2+ and control (VC) MC were treated for 1 h with 50 ng/ml PDGF to examine the effect of PDGF one the ROS generation in our cell culture system. Fig. 1D shows that PDGF did not influence ROS levels of COX-2+ MC. In contrast, as demonstrated in Fig. 1E, PDGF was capable of increasing ROS levels in control (VC) MC.

Quiescent COX-2+ and control (VC) MC were treated with 50 ng/ml PDGF or 5 μM menadione and with a combination of both substances in serum free RPMI-1640 media containing 2 μCi/ml [^{3}H] thymidine. Fig. 2A depicts the [^{3}H] thymidine incorporation values of COX-2+ and control (VC) MC after 24 h. While PDGF (1.31 ± 0.15-fold) or menadione (1.29 ± 0.13-fold) hardly affect cell proliferation of COX-2+ MC, the combined administration of PDGF and menadione significantly stimulate proliferation of this cell line 2.3 ± 0.25-fold ($n = 3$, $P < 0.05$). In control (VC) MC, 50 ng/ml PDGF or the elevation of the intracellular ROS levels through 5 μM menadione stimulate proliferation 2.11 ± 0.19-fold and 1.79 ± 0.09-fold, respectively. Both substances together additionally stimulate control (VC) MC proliferation 2.6 ± 0.87.

For cell counting, quiescent COX-2+ and control (VC) MC were treated again with 50 ng/ml PDGF or 5 μM menadione and with a combination of both substances in serum free RPMI-1640 media for 24 h. In Fig. 2B data from three independent experiments are shown. According to the cell proliferation assays (Fig. 2A) in COX-2+ MC PDGF (1.03 ± 0.02) and menadione (1.12 ± 0.07) hardly increased cell number of COX-2+ cells. In contrast, the combination of 50 ng/ml PDGF and 5 μM menadione increased cell number of COX-2+ MC (1.57 ± 0.07) after 24 h when compared to COX-2+ MC cultivated under serum free conditions. Furthermore, again in accordance to the cell proliferation assays (Fig. 2A) cell numbers of control (VC) MC showed comparable results. PDGF (1.68 ± 0.06) and menadione (1.48 ± 0.04) increased cell number and the combination of PDGF and menadione resulted

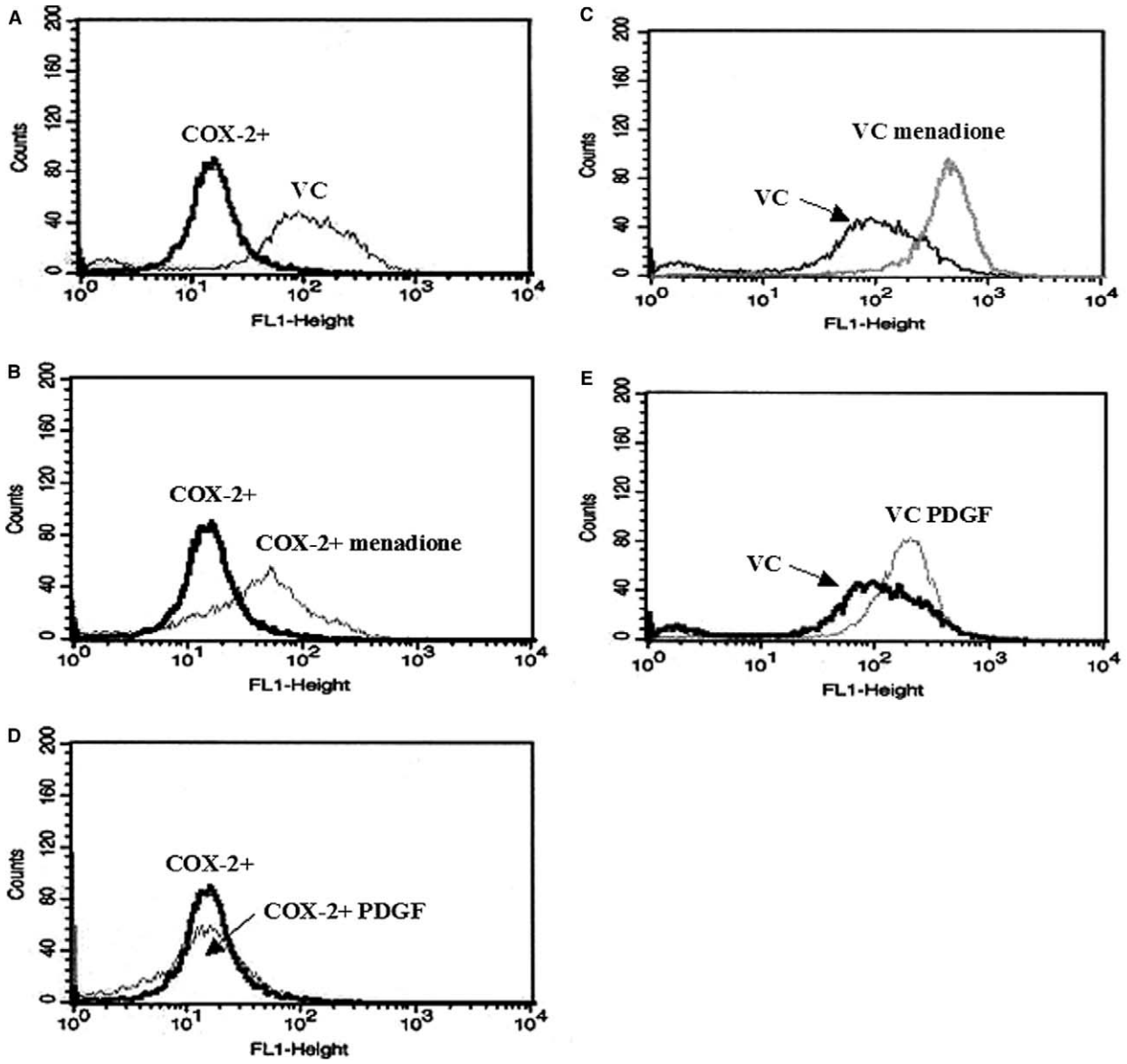

Fig. 1. The ROS levels of control (VC) MC and COX-2+ MC (COX-2+) were measured by ROS dependent hydrolysis of CM-H_2DCFDA and subsequent quantification of fluorescence. (A) Control (VC) MC strongly generated higher amounts of ROS than COX-2+ MC. (B,C) COX-2+ and control (VC) MC treated for 1 h with 5 μM menadione showed increased ROS generation compared to the appropriate cell-line in the absence of menadione. (D) The addition of 50 ng/ml PDGF for 1 h did not influence ROS generation of COX-2+ MC. (E) PDGF (50 ng/ml) for 1 h increased ROS generation of control (VC) MC. This graphs are representative for three independent experiments performed with qualitatively similar results.

in a further increase (1.97 ± 0.14) of the control (VC) MC after 24 h when compared to the appropriate cell line cultivated under serum free conditions.

To evaluate whether prostaglandins are responsible for the cell growth effects shown in Fig. 2, prostaglandin formation was inhibited with the non-specific COX-inhibitor indomethacin (1 μg/ml) for 48 h. In the last 24 h, 50 ng/ml PDGF, 5 μM menadione or a combination of both substances together with 2 μCi/ml [^{3}H] thymidine were added to all samples. As demonstrated in Fig. 3, neither PDGF (1.15 ± 0.3), menadione (0.99 ± 0.14), nor the combination of both substances (1.32 ± 0.32) significantly influenced proliferation of COX-2+ MC compared to the appropriate indomethacin treated cell line.

Recently, we demonstrated that COX-2 overexpression maintained gene expression of the CDK inhibitors $p21^{cip1}$ and $p27^{kip1}$ in the presence of PDGF and thus inhibits cell proliferation [11]. Since the intracellular increase of ROS restored PDGF-induced proliferation in COX-2+ MC, we further elucidated whether ROS influence protein expression of these CDK inhibitors. For this purpose Western-blot analysis of the CDK inhibitors $p21^{cip1}$ and $p27^{kip1}$ was performed. Quiescent COX-2+ and control (VC) MC were treated either with 50 ng/ml PDGF or 5 μM menadione and with the combination of both substances in serum free RPMI-1640 media for 1, 6 and 24 h. Protein expression of $p21^{cip1}$ as well as $p27^{kip1}$ was measured and compared with the protein levels of the appropriate cell line cultivated in serum free RPMI-1640 medium. As shown in Fig. 4, neither PDGF nor menadione alone significantly affected the protein expression of $p21^{cip1}$ and $p27^{kip1}$ in COX-2+ MC at all time points studied. In contrast, PDGF de-

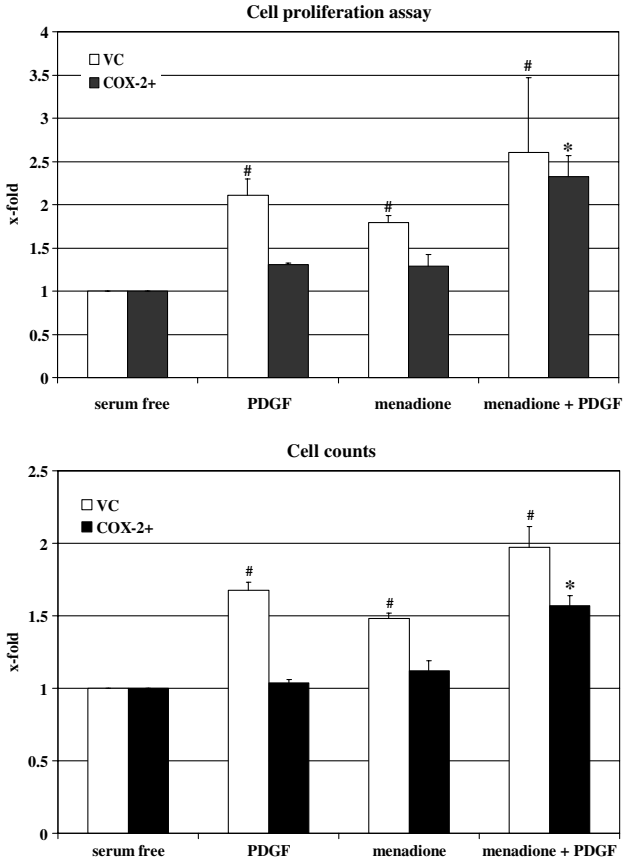

Fig. 2. Results of the cell proliferation 24 h after stimulation with the appropriate compounds. Serum free controls were assigned a relative value of one. The differences are shown in a *x*-fold manner vs. serum free control. (A) Results of [^{3}H] thymidine incorporation. The combined treatment of COX-2+ MC (COX-2+) with 5 μM menadione and 50 ng/ml PDGF significantly increased [^{3}H] thymidine incorporation when compared to the PDGF treatment alone ($n = 3$, $P^* < 0.05$; * vs. PDGF treatment). The treatment of either PDGF or menadione alone as well as the combination of both substances significantly increased [^{3}H] thymidine incorporation of control (VC) MC ($n = 3$, $P^{\#} < 0.05$; vs. serum free control) whereas [^{3}H] thymidine incorporation of COX-2+ MC hardly affect cell proliferation compared to the appropriate serum free control. (B) Results of the cell counts. In accordance to the cell proliferation assay in Fig. 1A the combined treatment of 5 μM menadione and 50 ng/ml PDGF significantly increased cell number in COX-2+ MC ($n = 3$, $P^* < 0.05$; * vs. PDGF treatment) compared to the appropriate PDGF treatment. Again, the treatment of either PDGF or menadione alone as well as the combination of both substances significantly increased the cell numbers of control (VC) MC ($n = 3$, $P^{\#} < 0.05$; # vs. serum free control) whereas the cell numbers of COX-2+ MC almost remained unaltered when compared to the appropriate serum free control.

creased the protein expression of p21^{cip1} and p27^{kip1} in control (VC) MC after one and 6 h whereas menadione alone hardly influenced p21^{cip1} and p27^{kip1} protein expression in this cell line. The combination of both substances strongly decreased protein expression of both CDK inhibitors after one and 6 h in COX-2+ as well as control (VC) MC. Since one single dose of 5 μM menadione was capable of increasing ROS levels in COX-2+ and control (VC) MC about 6–8 h (data not shown) and prolonged PDGF incubation leads to endogenous stimulation of COX-2 which consequently increases both CDK inhibitors, after 24 h the protein expression of p21^{cip1} as well as of p27^{kip1} was almost restored in both cell lines.

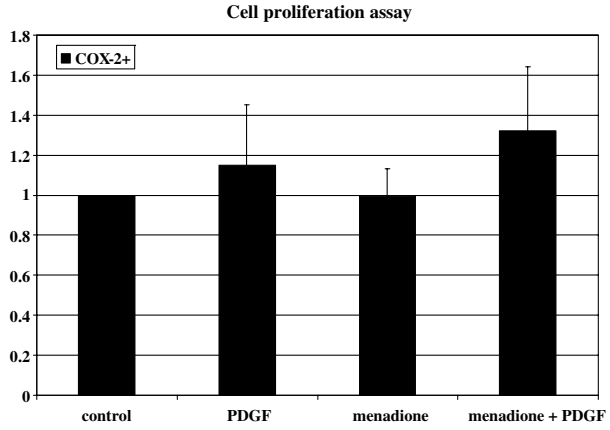

Fig. 3. Effect of COX-2 inhibition on cell proliferation ($n = 3$). COX-2+ MC were incubated for 48 h in serum free media in the presence of 1 μg/ml indomethacin. In the last 24 h, 50 ng/ml PDGF, 5 μM menadione or a combination of both substances together with 2 μCi/ml [^{3}H] thymidine were added to all samples. The different treatments were normalized to indomethacin treated COX-2+ MC. Neither PDGF, menadione nor the combination of both substances significantly influenced cell proliferation compared to indomethacin treated COX-2+ MC.

4. Discussion

The proliferation of glomerular MC is a hallmark in immune mediated renal diseases such as GN [1]. The formation of the cyclooxygenase product prostaglandin E_2 [14–17] as well as the generation of ROS are enhanced in these diseases [3–6]. Since it has been shown that in airway smooth muscle cells ROS are required for PDGF-induced growth [7] and the overexpression of COX-2 exerts antiproliferative effects in glomerular MC [11] which may counteract growth promoting events in glomerular injury, the potential role of ROS in the COX-2 dependent cell cycle arrest was examined in the current study.

The ROS concentrations of COX-2+ MC were decreased when compared to control (VC) MC which implies a potential COX-2 dependent influence on the cellular redox system either by downregulation of the NADPH oxidase complex, which is the major source of intracellular ROS and could influence cell cyle regulation [22], and/or upregulation of enzymes such as SOD and GPX which are capable to metabolize ROS in H_2O and O_2. Moreover, catalase and NADPH oxidase could probably be influenced by COX-2 because increased catalase activity blocked PDGF-induced processes [19] and NADPH oxidase plays a crucial role in PDGF-induced proliferation in other cell systems [23].

Since PDGF increases the generation of ROS [19] and in our control (VC) MC but not in COX-2+ MC (Fig. 1D, E), COX-2 seems to prevent the PDGF-induced ROS generation and thus inhibits the mitogenic signal normally induced through PDGF. Moreover, exogenous administration of the ROS donor menadione increases intracellular ROS concentration in COX-2+ and control (VC) MC but replaced PDGF-induced proliferation only in control (VC) MC (Fig. 2). Thus, beside the effects on the cellular redox system, COX-2 seems to interfere with multiple cellular signaling pathways. This may include the phosphatidylinositol 3-kinase pathway which could mediate PDGF-induced ROS production [21] as well as ROS dependent downstream signaling pathways such as the erk1/2 MAP-kinase pathway [13,18]. The COX-2 induced growth inhibition could only be restored if PDGF and menadione were given in combination to

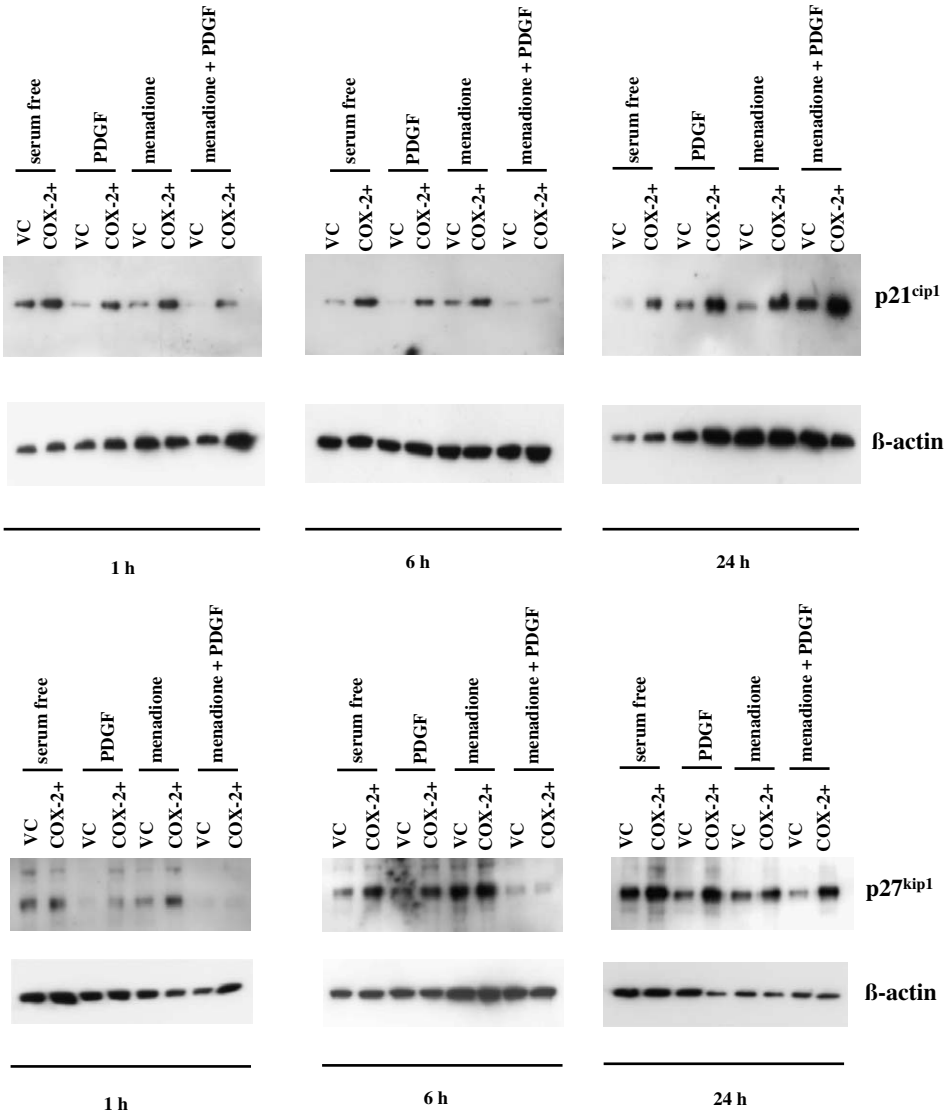

Fig. 4. Western blot for the CDK inhibitors $p21^{cip1}$ (A) and $p27^{kip1}$ (B) of COX-2+ and control (VC) MC. PDGF (50 ng/ml) decreased protein expression of both CDK inhibitors in control (VC) MC after one and 6 h but did not significantly influence these protein levels in COX-2+ MC. The combined treatment with 5 μM menadione and 50 ng/ml PDGF strongly decreased protein expression of the CDK inhibitors for one and 6 h in both cell lines. After 24 h the protein expression of $p21^{cip1}$ and $p27^{kip1}$ are almost restored in both cell lines. These blots are representative for three independent experiments performed with qualitatively similar results.

COX-2+ MC. Thus, both substances together bypass the influence of COX-2 leading to PDGF-induced cell proliferation. It seems that a distinct amount of intracellular available ROS are necessary to bypass the COX-2 inhibited cellular signaling pathways through PDGF in COX-2+ MC.

Since prostaglandins are known to influence MC proliferation [25] the potential role of these cyclooxygenase products on ROS dependent cell proliferation in COX-2+ MC were examined. COX-2+ MC were treated with the non-specific COX inhibitor indomethacin (1 μg/ml) for 48 h. Although 1 μg/ml indomethacin almost completely inhibits COX activity in COX-2+ MC [11] neither PDGF or menadione nor the combination of both are capable of significantly inducing proliferation. This observation is in accordance with earlier studies of others [26] and ourselves [11] which showed almost no influence of COX inhibition in cells stable overexpressing COX-2.

Interestingly, ROS and PDGF require almost 24 h to bypass the COX-2 induced cell cycle arrest (Fig. 2), which supposes indirect mechanisms probably through influencing factors like CDK inhibitors of the kip/cip family in the G1 phase of the cell cycle. Recently, we have demonstrated the importance of the CDK inhibitors $p21^{cip1}$ and $p27^{kip1}$ in the COX-2 dependent cell cycle arrest [11] and others have demonstrated that oxidative stress could influence $p21^{cip1}$ degradation in cystic fibrosis lung epithelial cells [24]. Therefore, we examined the possible influence of ROS on the protein expression of both CDK inhibitors. In the presence of menadione PDGF strongly decreased protein expression of $p21^{cip1}$ as well as $p27^{kip1}$ in COX-2+ MC within 1 h when compared to the same cell line in the absence of a ROS donor. Again, the elevation of the intracellular ROS levels due to the menadione administration in COX-2+ MC in combination with PDGF prevents the COX-2 dependent stabilization of $p21^{cip1}$ and $p27^{kip1}$ beginning within 1 h which consequently leads to proliferation of COX-2+ MC after 24 h. This fast downregulation of both CDK inhibitors further supports the participation of post-

translational protein modifications in cellular signaling pathways rather than events leading to new protein synthesis. Phosphorylation, for example, of protein kinases within the phosphatidylinositol 3-kinase and/or erk1/2 MAP-kinase pathways in response to PDGF and menadione could be responsible for the fast degradation of $p21^{cip1}$ and $p27^{kip1}$. 24 h after PDGF and menadione administration the protein expression of both CDK inhibitors is almost restored. This effect was expected since one single dose of 5 μM menadione was capable of increasing ROS levels in COX-2+ and control (VC) MC about 6–8 h (data not shown). Furthermore, prolonged PDGF incubation leads to endogenous stimulation of COX-2 which consequently increases both CDK inhibitors studied after 24 h.

In contrast, Hannken et al. [13] have shown an induction of $p27^{kip1}$ by H_2O_2 in proximal tubular cells leading to hypertrophy of these cells. Furthermore, Macip et al. [20] have demonstrated that $p21^{cip1}$ increased intracellular ROS which induced senescence in normal fibroblasts and p53-negative cancer cells. ROS inhibition rescued $p21^{cip1}$ induced senescence. These differences between our study where increased intracellular ROS clearly mediate proliferation through downregulation of $p27^{kip1}$ as well as $p21^{cip1}$ and the studies of Hannken and Macip could possibly be attributed to the use of different cell types. Furthermore, it could be possible that the increase of intracellular ROS through exogenously administered ROS donors downregulates $p21^{cip1}$ in a negative feedback regulation loop. No doubt, this discrepancy needs further clarification.

In conclusion, the data presented in the current study reveal ROS as important mediators of MC proliferation. ROS seem to function as competence factors which must be present at a specific concentration in order to permit PDGF-induced cell proliferation. COX-2 might therefore influence proliferation of glomerular MC through controlling ROS levels. Since ROS levels are increased in proliferative renal disease this study underscores the role of ROS in controlling proliferative events. Thus, modulation of the ROS levels in an early phase of such disease might possibly exert beneficial effects on the progression of renal diseases.

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References

[1] Johnson, R.J. (1994) The glomerular response to injury: progression or resolution. Kidney Int. 45, 1769–1782.
[2] Johnson, R.J., Floege, J., Couser, W.G. and Alpers, C.E. (1993) Role of platelet-derived growth factor in glomerular disease. J. Am. Soc. Nephrol. 4, 119–128.
[3] Gwinner, W. and Groene, H.J. (2000) Role of reactive oxygen species in glomerulonephritis. Nephrol. Dial. Transplant. 15, 1127–1132.
[4] Rohrmoser, M.M. and Mayer, G. (1996) Reactive oxygen species and glomerular injury. Kidney Blood Press. Res. 19, 263–269.
[5] Shah, S.V. (1995) The role of reactive oxygen metabolites in glomerular disease. Annu. Rev. Physiol. 57, 245–262.
[6] Budisavljevic, M.N., Hodge, L.A., Barber, K., Fulmer, J.R., Durazo-Arvizu, R.A., Self, S.E., Kuhlmann, M., Raymond, J.R. and Greene, E.L. (2003) Oxidative stress in the pathogenesis of experimental mesangial proliferative glomerulonephritis. Am. J. Physiol. 285, F1138–F1148.
[7] Brar, S.S., Kennedy, T.P., Whorton, A.R., Murphy, T.M., Chitano, P. and Hoidal, J.R. (1999) Requirement for reactive oxygen species in serum-induced and platelet-derived growth factor-induced growth of airway smooth muscle. J. Biol. Chem. 274, 20017–20026.
[8] Herschman, H.R. (1996) Prostaglandin synthase-2. Biochim. Biophys. Acta 1299, 125–140.
[9] Warner, T.D., Guiliano, F., Vojnovic, I., Bukasa, A., Mitchell, J.A. and Vane, J.R. (1999) Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc. Natl. Acad. Sci. 96, 7563–7568.
[10] Dubois, R.N., Abramson, S.B., Crofford, L., Gupta, R.A., Simon, L.S., Nan de Putte, L.B. and Lipsly, P.E. (1998) Cyclooxygenases in biology and disease. FASEB J. 12, 1063–1073.
[11] Zahner, G., Wolf, G., Ayoub, M., Reinking, R., Panzer, U., Shankland, S.J. and Stahl, R.A.K. (2002) Cyclooxygenase-2 overexpression inhibits platelet-derived growth factor- induced mesangial cell proliferation through induction of the tumor suppressor gene p53 and the cyclin-dependent kinase inhibitors $p21^{waf1/cip1}$ and $p27^{kip1}$. J. Biol. Chem. 277, 9763–9771.
[12] Baud, L., Fouqueray, B., Philippe, C. and Ardaillou, R. (1992) Reactive oxygen species as glomerular autacoids. J. Am. Soc. Nephrol. 2 (Suppl. 2), S132–S138.
[13] Hannken, T., Schroeder, R., Zahner, G., Stahl, R.A.K. and Wolf, G. (2000) Reactive oxygen species stimulate p44/42 mitogen-activated protein kinase and induce $p27^{kip1}$: Role in angiotensin II-mediated hypertrophy of proximal tubular cells. J. Am. Soc. Nephrol. 11, 1387–1397.
[14] Lianos, E.A., Andres, G.A. and Dunn, M.J. (1983) Glomerular prostaglandin and thromboxane synthesis in rat nephrotoxic serum nephritis. Effects on renal hemodynamics. J. Clin. Invest. 72, 1439–1448.
[15] Stahl, R.A.K., Adler, S., Baker, P.J., Chen, Y.P., Protzl, P.M. and Couser, W.G. (1987) Enhanced glomerular prostaglandin formation in experimental membranous nephropathy. Kidney Int. 31, 1126–1131.
[16] Stork, J.E. and Dunn, M.J. (1985) Hemodynamic roles of thromboxane A2 and prostaglandin E2 in glomerulonephritis. J. Pharmacol. Exp. Ther. 233, 672–678.
[17] Stahl, R.A.K., Kudelka, S., Paravicini, M. and Schollmeyer, P. (1986) Prostaglandin and thromboxane formation in glomeruli from rats with reduced renal mass. Nephron 42, 252–257.
[18] Abe, M.K., Chao, T.S.O., Solway, J., Rosner, M.R. and Hershenson, M.B. (1994) Hydrogen peroxide stimulates mitogen-activated protein kinase in bovine tracheal myocytes: Implications for human airway disease. Am. J. Respir. Cell Mol. Biol. 11, 577–585.
[19] Sundaresan, M., Zu-Xi, Y., Ferrans, V.J., Irani, K. and Finkel, T. (1995) Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. Science 270, 296–299.
[20] Macip, S., Igarashi, M., Fang, L., Chen, A., Pan, Z.-Q., Lee, S.W. and Aaronson, A. (2002) Inhibition of p21-mediated ROS accumulation can rescue p21-induced sensescence. EMBO J. 21, 2180–2188.
[21] Bae, Y.S., Sung, J.-Y., Kim, O.-S., Kim, Y.J., Hur, K.C., Kazlauskas, A. and Rhee, S.G. (2000) Platelet-derived growth factor-induced H_2O_2 production requires the activation of phosphatidylinositol 3-kinase. J. Biol. Chem. 275, 10527–10531.
[22] Berasi, S.P., Xiu, M., Yee, A.S. and Paulson, K.E. (2004) HBP1 repression of the p47phox gene: cell cycle regulation via the NADPH oxidase. Mol. Cell. Biol. 24, 3011–3024.
[23] Adachi, T., Togashi, H., Suzuki, A., Kasai, S., Ito, J., Sugahara, K. and Kawata, S. (2005) NADP(H) oxidase plays a crucial role in PDGF-induced proliferation of hepatic stellate cells. Hepatology 41, 1272–1281.
[24] Boncoeur, E., Tabary, O., Bonvin, E., Muselet, C., Fritah, A., Lefait, A., Lefait, E., Redeuilh, G., Clement, A., Jacquot, J. and Henrion-Caude, A. (2006) Oxidative stress response results in increased p21(WAF1/CIP1) degradation in cystic fibrosis lung epithelial cells. Free Radic. Biol. Med. 40, 75–86.
[25] Stahl, R.A., Thaiss, F., Haberstroh, U., Kahf, S., Shaw, A. and Schoeppe, W. (1990) Cyclooxygenase inhibition enhances rat interleukin 1 beta-induced growth of rat mesangial cells in culture. Am. J. Physiol. 259, F419–F424.
[26] Trifan, O.C., Smith, R.M., Thompson, B.D. and Hla, T. (1999) Overexpression of cyclooxygenase-2 induces cell cycle arrest: Evidence for a prostaglandin-independent mechanism. J. Biol. Chem. 274, 34141–34147.